

RANDOM TRUNCATION AND AMPLIFICATION OF NUCLEIC ACID

ABSTRACT

5 A method is provided for producing a library of mutagenized polynucleotides from a target sequence comprising (a) taking a sample comprising: (i) a target sequence including a section to be mutagenized, (ii) a library of first primers where the first primers include a first fixed sequence and a first unknown sequence 3' to the first fixed sequence, 10 the first unknown sequence varying within the library of first primers, and (iii) a library of second primers where the second primer include a second fixed sequence that differs from the first fixed sequence, and a second unknown sequence 3' to the second fixed sequence, the second unknown sequence varying within the library of second primers; (b) 15 performing one or more cycles of primer extension amplification on the sample in the presence of at least one polymerase such that a member of the library of the first primers is extended relative to the target sequence; and (c) performing one or more additional cycles of primer extension amplification on the sample such that a member of the library 20 of the second primers is extended relative to the first primer that was extended in step (b) to form the library of mutagenized polynucleotides. The mutagenesis produces a library of mutagenized targeted sequences with random truncations.